FISHERY RESEARCH

NATIVE SPECIES INVESTIGATIONS

Grant # F-73-R-25

July 1, 2003 to June 30, 2004

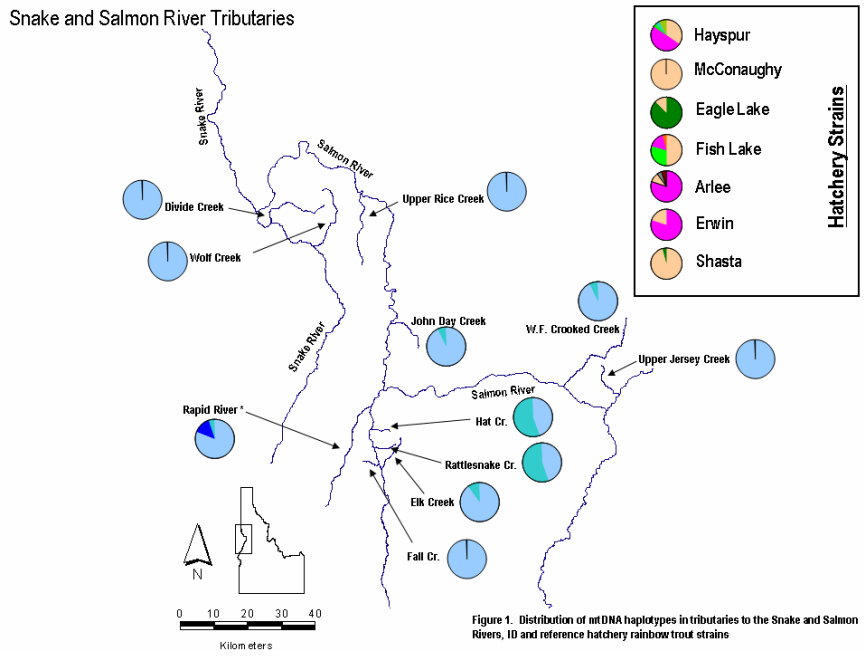

Figure 1. Distribution of mtDNA haplotypes in tributaries to the Snake and Salmon Rivers, ID and reference hatchery rainbow trout strains

Matthew Campbell
Sr. Fisheries Research Biologist

Christine Cegelski
Fisheries Genetics Research Assistant

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Annual Performance Report

July 1, 2003 to June 30, 2004

Grant # F-73-R-25

Project 2: Native Species Investigations

By

Matthew Campbell
Christine Cegelski

Idaho Department of Fish and Game
600 South Walnut Street
P.O. Box 25
Boise, ID 83707

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

List of Figures, continued.

Page

LIST OF APPENDICES

PREFACE

Since the spring of 2002, Idaho Department of Fish and Game (IDFG) has operated a fish genetics laboratory at the Eagle Fish Hatchery to provide an efficient, cost-effective means of generating detailed genetic information necessary for the improved management and conservation of Idaho's native fish species. This report describes two research projects completed by the lab during the July 1, 2003 to June 30, 2004 contract period. The first project describes collaborative research with the Bureau of Land Management examining the genetic relatedness and purity of 10 allopatric redband trout populations within the Snake and Salmon River drainages. These two research topics (relatedness and purity) involving resident *Oncorhynchus mykiss* populations are particularly important because the National Marine Fisheries Service (NMFS) is currently proposing to amend existing Endangered Species Act (ESA) listing determinations for some resident *O. mykiss* populations (Federal Register, Vol. 69, No. 113, 2004). Tentatively, the NMFS has ruled that Evolutionarily Significant Unit (ESU) or Distinct Population Segment (DPS) membership of resident *O. mykiss* populations (whether to include or not include them in the same ESU or DPS as geographically approximate anadromous populations) will depend on whether they fit into one of three different categories: 1) pure allopatric resident *O. mykiss* populations, isolated above long-standing natural barriers *will not be* included in the same ESU with *O. mykiss* populations below these barriers, 2) resident *O. mykiss* populations that exist sympatrically with anadromous *O. mykiss* (not separated by a geographic barrier) *will be* included in the same ESU as the anadromous *O. mykiss* population, 3) the listing determination for resident *O. mykiss* populations that are isolated above man-made barriers (dams) from anadromous *O. mykiss* populations will be evaluated on a case-by-case basis. Currently, NMFS has proposed that populations of resident *O. mykiss* in the North Fork Clearwater, above Dworshak Dam, should be included in the same ESU as anadromous *O. mykiss* populations below the dam. This decision was based on earlier genetic work suggesting that *O. mykiss* above and below the dam share a recent common ancestry (Federal Register, Vol. 69, No. 113, 2004).

Complicating the above North Fork Clearwater determination, as well as most current listing determination decisions, is that little or no genetic information regarding intraspecific hybridization (hybridization with introduced hatchery rainbow trout) is available for most resident *O. mykiss* populations (Federal Register, Vol. 69, No. 113, 2004).

The research described in this first research subproject provides some preliminary information regarding the genetic status of resident allopatric *O. mykiss* populations in the Salmon and Snake River drainages. Results from this study should assist managers in making prioritization and conservation decisions regarding these populations and provide perspective on current and future ESA decisions proposed by NMFS.

The second project described in this report is an investigation of rainbow trout hybridization within several westslope cutthroat trout *O. clarkii lewisi* populations in the Coeur d'Alene River Basin, Idaho. Hybridization issues concerning westslope cutthroat trout and rainbow trout remain priorities for IDFG. In addition to IDFG priorities, legal and scientific battles exist over the question of whether westslope cutthroat trout should be listed as a threatened species under the Endangered Species Act. It is unclear whether hybrids between the two species and hybridized populations should be included as westslope cutthroat trout in the unit considered for listing. In the fall of 2003, the United States Fish and Wildlife Service (USFWS) upheld its previous 2000 decision that westslope cutthroat trout do not warrant listing and concluded that it was appropriate under specific circumstances to include a westslope cutthroat

trout population introgressed with up to 20% rainbow trout genes in the unit considered for listing (Federal Register, Vol. 68, No. 152, 2004).

While research focused on assessing introgression within westslope cutthroat trout populations is a priority for IDFG and will be important for future status assessments, this second research subproject was not designed to provide precise estimates of rainbow trout introgression levels. Instead, this work deals more with the practical issues associated with minimizing the threat of hybridization and introgression to westslope cutthroat trout populations. Current management strategies for reducing or eliminating hybrids and self-reproducing populations of introduced nonnative rainbow trout include changes to fishing regulations to allow for the harvest of hybrids and rainbow trout and the use of weirs on spawning tributaries to remove migrating hybrids and rainbow trout from spawning populations (Host 2003). Both of these strategies rely on the ability of biologists to use phenotype-based characters to accurately distinguish cutthroat trout from hybrids and rainbow trout and are the primary focus of this second research subproject.

Two additional research projects not included in this document need to be mentioned. Another genetic project finished during the July 1, 2003 to June 30, 2004 contract period was an investigation of hybridization between bull trout and brook trout in the Upper North Fork Clearwater Basin. Results from this work were published in a Regional Fisheries Management Report (Schriever et al. 2004).

A second project worked on during the contract period but not completed was an investigation of hybridization and genetic population structure of Yellowstone cutthroat trout *O. clarkii bouvieri* throughout their range in Idaho (Snake River Native Salmonid Assessment, BPA project # 199800200). Findings from this work were presented at the Idaho, Western, and National American Fisheries Society Meetings in 2004. Currently, three papers are in preparation for submission to the Transactions of the American Fisheries Society. Genetic results from this project will also be included in next year's Annual Completion Report.

JOB PERFORMANCE REPORT
SUBPROJECT #1: MITOCHONDRIAL DNA ANALYSIS OF REDBAND TROUT *ONCORHYNCHUS MYKISS GAIRDNERI* FROM TRIBUTARIES TO THE SALMON AND SNAKE RIVERS, IDAHO

State of: Idaho Grant No.: F-73-R-25, Fishery Research

Project No.: 2 Title: Native Species Investigations

Subproject #1: Mitochondrial DNA analysis of redband trout *Oncorhynchus mykiss gairdneri* from tributaries to the Salmon and Snake rivers, Idaho

Contract Period: July 1, 2003 to June 30, 2004

ABSTRACT

The identification and conservation of pure, native redband trout populations are goals of both the Bureau of Land Management and the Idaho Department of Fish and Game. Of particular conservation interest are redband trout populations found above natural barriers. In many cases, these populations are protected from upstream invasion of exotic introduced trout. Additionally, previous studies have suggested that many of these allopatric populations exhibit substantial genetic and life history divergence from their downstream counterparts, thus making them potentially unique and important components of the overall genetic diversity of *O. mykiss*. In this study, 10 populations of *O. mykiss* isolated above migration barriers in the Snake and Salmon river drainages were sampled and compared to one downstream, native anadromous *O. mykiss* population and to six nonnative hatchery rainbow trout populations. Mitochondrial DNA analyses were used to examine genetic relationships between populations and to assess intraspecific hybridization. Results provide evidence that these populations are pure native interior redband trout. No mitochondrial haplotypes observed in six reference hatchery rainbow trout populations/strains were observed in any of these populations. Mitochondrial haplotypes were shared between the 10 allopatric redband trout populations as well as one steelhead population. These preliminary findings suggest that these populations should be prioritized for conservation and likely managed independently from *O. mykiss* populations below these barriers.

Authors:

Matthew Campbell
Fisheries Research Biologist

Christine Cegelski
Fisheries Genetics Research Assistant

INTRODUCTION

Interior Columbia River redband trout *Oncorhynchus mykiss gairdneri* in Idaho exhibit two life history forms: anadromous (steelhead) and nonanadromous (resident). The anadromous form is currently listed as threatened under the ESA (August 18, 1997, 62 Federal Register 43974). The nonanadromous form, which is the focus of this study, can be further divided into those sympatric with or allopatric with steelhead. Sympatric redband trout reside in the same area as steelhead but have developed separate, although not completely reproductively isolated, populations due to differences in life history characteristics (Currens et al. 1997). Allopatric redband trout are populations that have been historically isolated from steelhead upstream of full fish passage barriers. It has been hypothesized that these populations may be evolutionarily distinct from both the resident and anadromous populations downstream of these barriers (Quigley et al. 1997), and it has been suggested that these populations should be included in their own Evolutionarily Significant Units (ESUs) or Distinct Populations Segments (DPSs) when making future decisions related to ESA listings (Kostow 2003).

While several studies have demonstrated large genetic differences between populations of *O. mykiss* above and below natural barriers (Currens et al. 1990; Currens et al. 1997; Phelps et al. 1998), interpreting these differences from an evolutionary significance perspective is complicated. These populations are isolated and often very small, and their genetic variation and structure has likely been strongly influenced by genetic drift (Kostow 2003). An additional complication in assessing the genetic diversity and uniqueness of redband trout populations throughout Idaho is that many areas have been stocked with hatchery rainbow trout, which has resulted in intraspecific hybridization and introgression, altering natural genetic variation and structure.

The purpose of this preliminary study is to assess the genetic relatedness and purity of allopatric redband trout populations within the Snake and Salmon river drainages in Idaho. We chose mitochondrial DNA (mtDNA) analysis for this assessment because its high mutation rate, strict maternal inheritance, and low effective population size makes it a useful tool for investigating the population structure of recently diverged or closely related groups of taxa (Avise 1994). Additionally, the utility of mtDNA Restriction Fragment Length Polymorphism (RFLP) analysis in assessing intraspecific O. *mykiss* hybridization has been previously demonstrated. Williams and Jaworski (1995) and Williams et al. (1996) examined mtDNA diversity in native trout populations from the Kootenai River in northern Idaho and from several native and nonnative trout populations in southern Idaho. They concluded that pure, native redband trout populations typically exhibit only one or two mtDNA haplotypes that differ only slightly from one another, usually less than 0.5% sequence divergence. In contrast, redband trout populations that have interbred with hatchery rainbow trout often exhibit multiple mtDNA haplotypes that differ from one another by up to 1.5-2.2% sequence divergence.

OBJECTIVES

1. Assess the genetic relatedness of 10 allopatric redband trout populations within the Snake and Salmon river drainages in Idaho.

2. Assess the genetic purity of 10 allopatric redband trout populations within the Snake and Salmon river drainages in Idaho.

METHODS

Sample Collection

Nonlethal fin clip samples were collected from *O. mykiss* via hook and line and/or a backpack electroshocker sampling in 10 tributaries of the Snake and Salmon river drainages (Figure 1; Table 1). These sites were chosen because, while in the general range of steelhead, each occurred above a full passage barrier. One steelhead population (Rapid River) was also sampled from the Salmon River drainage for use as a reference population of the anadromous life history form.

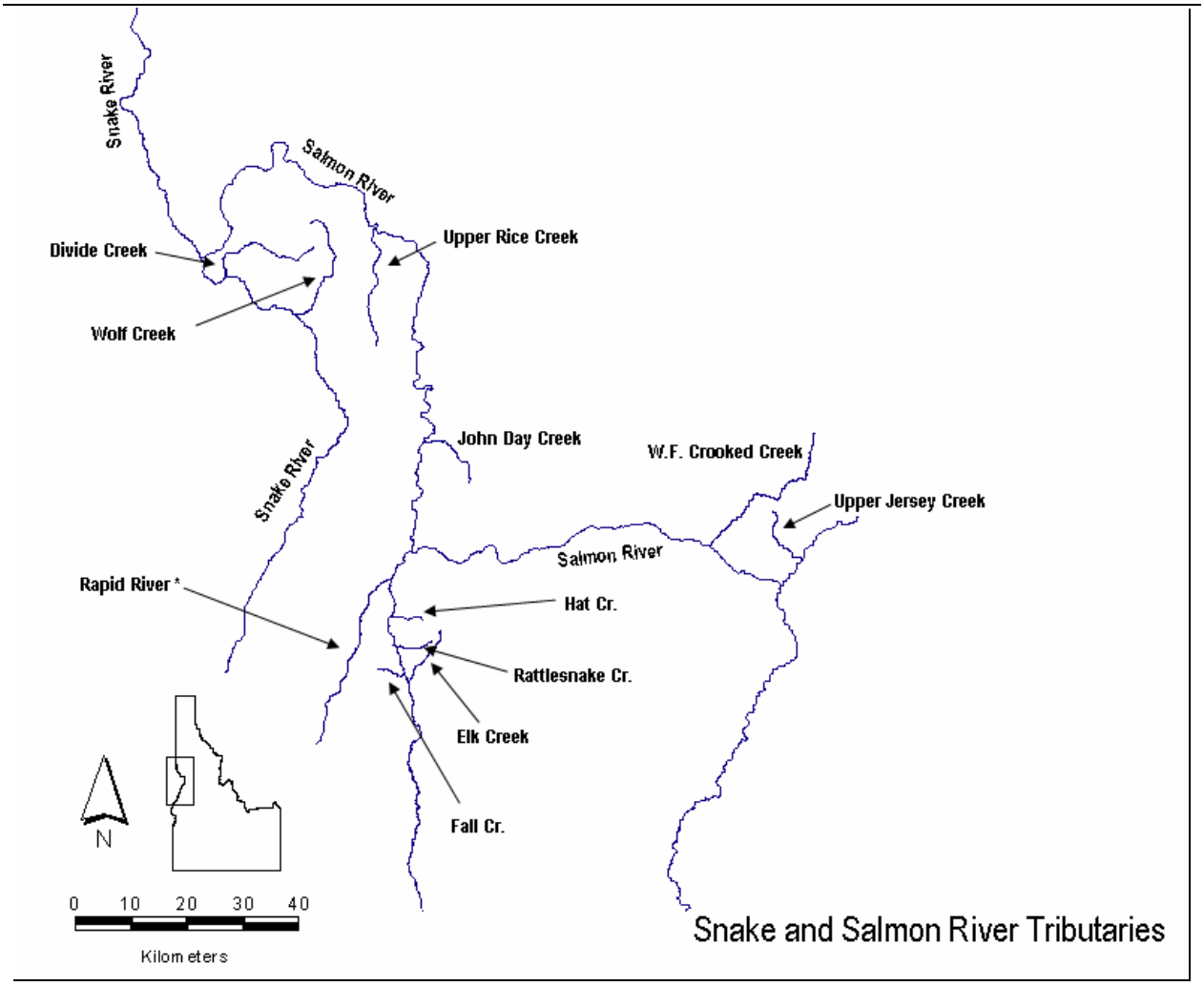

Figure 1. Sample locations in tributaries to the Snake and Salmon rivers, Idaho including the Rapid River reference population.

Table 1. Drainage, sample location, and sample size for samples collected from the Snake and Salmon rivers, Idaho.

Drainage	Location	N
Salmon River	John Day Creek	30
Salmon River	Upper Rice Creek	30
Salmon River	Upper Jersey Creek	30
Salmon River	WF Crooked Creek	30
Little Salmon River	Elk Creek	30
Little Salmon River	Hat Creek	30
Little Salmon River	Rattlesnake Creek	30
Little Salmon River	Fall Creek	30
Little Salmon River	Rapid River*	24
Snake River	Wolf Creek	30
Snake River	Divide Creek	30

* Samples from Rapid River came from adult wild steelhead and were used for comparison purposes.

Samples from seven hatchery rainbow trout strains were analyzed for comparison purposes (Table 2). Samples from Fish Lake, Eagle Lake, Arlee, McConaughy, Erwin, and Shasta strains were chosen because the DNA had already been isolated, and they represent a diverse collection of many of the primary rainbow trout hatchery strains that have been stocked throughout the Western States during the last 100 years. The Hayspur Hatchery strain was chosen because the strain originated in Idaho and is an admixture of several commercial and indigenous rainbow trout strains (Williams et al. 1996). The Hayspur strain has been one of the most stocked hatchery rainbow trout strains in Idaho during the past decade (http://www2.state.id.us/fishgame/fish/fishstocking/stocking/index.cfm).

Table 2. Hatchery source and sample size for rainbow trout strains analyzed.

Hatchery Source	Strain	N
Ennis NFH, Montana	Fish Lake	24
Ennis NFH, Montana	Eagle Lake	24
Ennis NFH, Montana	Arlee	24
Ennis NFH, Montana	McConaughy	24
Ennis NFH, Montana	Erwin	24
Ennis NFH, Montana	Shasta	24
Nampa FH, Idaho	Hayspur	30

Genetic Analysis

Total genomic DNA was extracted from a 1 x 1 mm piece of fin clip following methods described by Campbell (2000). DNA was resuspended in 100 µl TE. Six samples from each of the hatchery rainbow trout reference populations and six samples from Wolf Creek, Upper Rice Creek, Divide Creek, and Fall Creek were amplified using the Polymerase Chain Reaction

(PCR) with primers specific for the combined NADH Dehydrogenase 1 and 2 gene regions of the mitochondrial genome (~3500 b.p.). Amplification products were subsequently digested with 11 restriction enzymes (*Alu-I, Bfa-I, Dpn-II, Dde-I, Hae-III, Hha-I, Hinf-I, Mse-I, Msp-I, Rsa-I* and *Taq-I*) and electrophoresed on 3% agarose gels to screen for genetic differences between populations. Of these 11 restriction enzymes, four that produced informative polymorphic differences between populations (*Hae-III, Hha-I, Hinf-I, and Mse-I*) were chosen for analysis on all remaining samples (Examples: Figures 2 and 3).

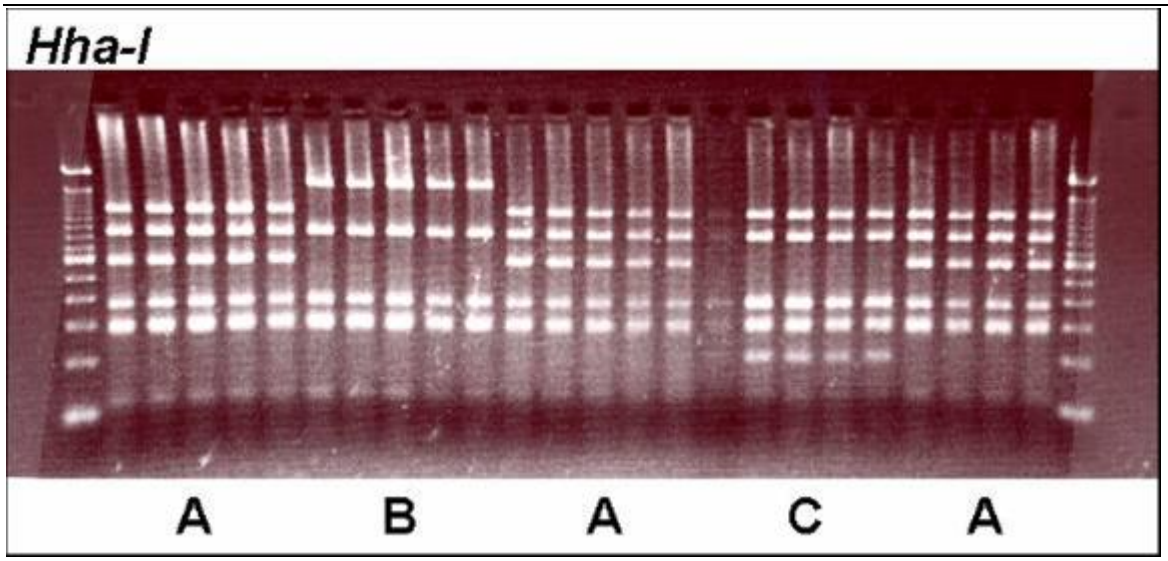

Figure 2. *Hha-I* digest demonstrating polymorphic banding patterns. Each unique banding pattern (polymorphism) generated by a specific enzyme is assigned a letter.

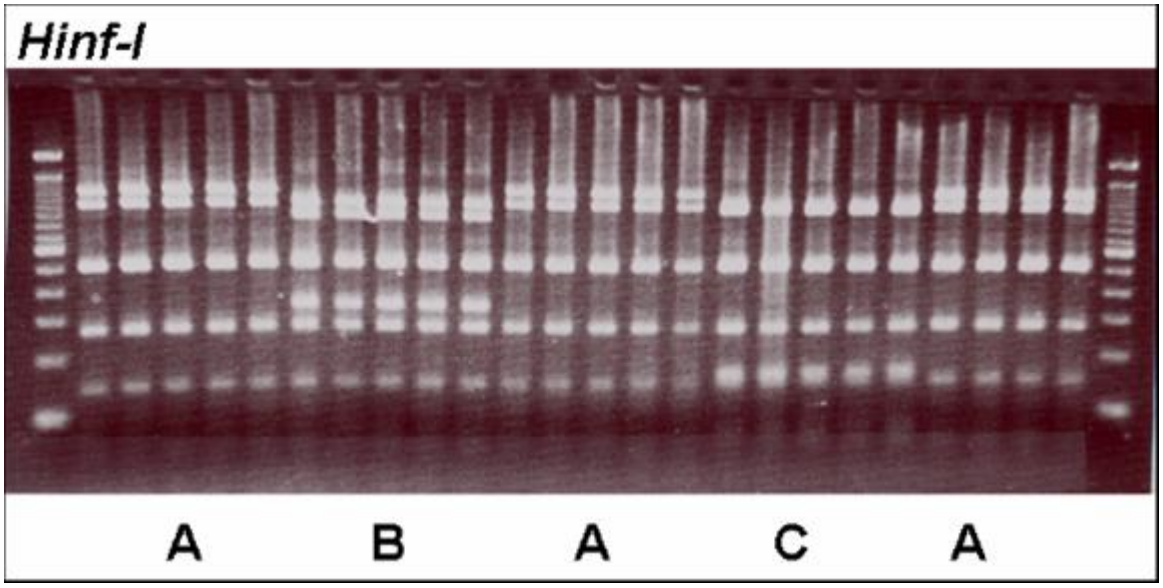

Figure 3. *Hinf-I* digest demonstrating polymorphic banding patterns. Each unique banding pattern (polymorphism) generated by a specific enzyme is assigned a letter.

Polymorphisms “A” and “C” observed in *Hae-III* digests were particularly difficult to distinguish on 3% agarose gels (Figure 4). To ensure that accurate calls were made, the majority of *Hae-III* digests were run on higher resolution 6% polyacrylamide gels (Figure 5).

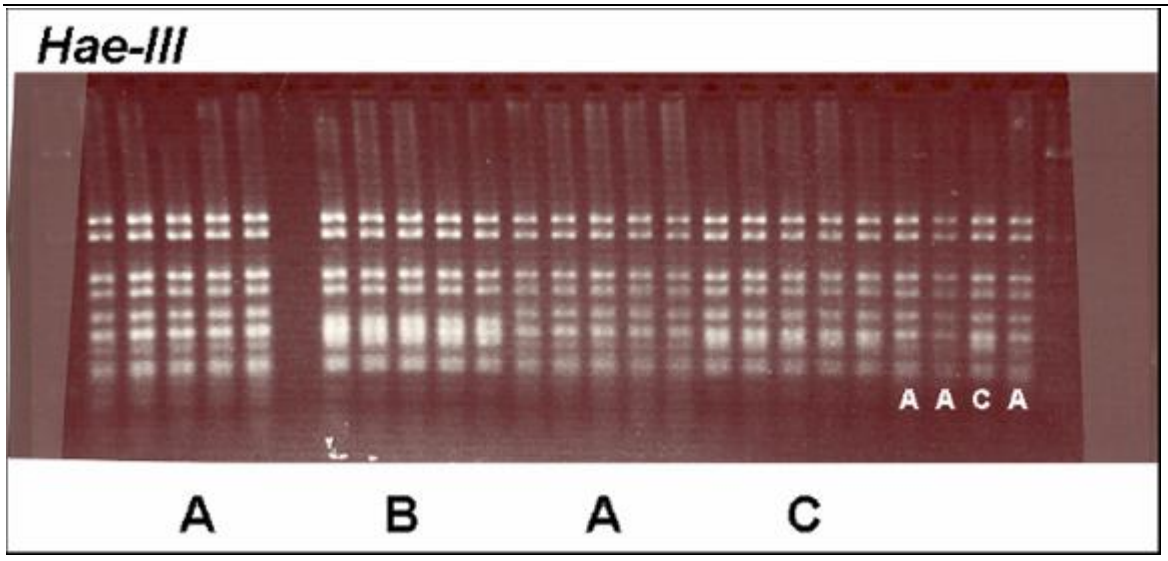

Figure 4. *Hae-III* digest demonstrating polymorphic banding patterns on 3% agarose gel.

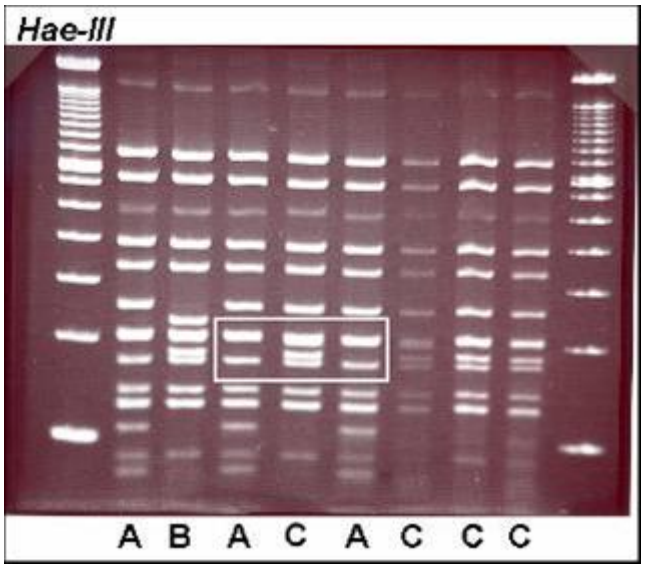

Figure 5. *Hae-III* digest demonstrating polymorphic banding patterns on 6% polyacrylamide gel.

As illustrated above, each unique banding pattern (polymorphism) generated by a specific restriction enzyme was assigned a letter. The letter designations for each of the four restriction enzymes were later combined across enzymes to form a composite haplotype.

Haplotypes and haplotype frequencies were compared between samples collected from the 10 Snake and Salmon river tributary populations and the hatchery rainbow trout reference population. The genetic software programs REAP (McElroy et al. 1991) and PHYLIP version 3.53 (Felsenstein 1993) were used to generate estimates of genetic divergence among haplotypes and to construct a Least Squares dendogram.

RESULTS

We obtained complete haplotypes from 255 samples from the 10 isolated Snake and Salmon river tributary populations (Table 3, Figure 6). Only two haplotypes were observed among all of the sample locations (haplotypes A and B). Sequence divergence between the two haplotypes was 0.40% (Table 4). Haplotype A was most common haplotype, occurring in all sample sites and in 84.7% of the samples. Haplotype B was observed in only half the sites, but was the more frequent of the two haplotypes at two sites (Rattlesnake Creek-57% and Hat Creek-55%).

The one steelhead reference population examined in this study (Rapid River) exhibited three haplotypes. Both haplotypes observed in the redband populations above barriers were observed in samples from Rapid River (A-81%, B-5%). An additional haplotype was also observed (D-14%). Sequence divergence among the three haplotypes ranged from 0.40%-0.79%.

Large differences in haplotype frequency and diversity was observed among the hatchery rainbow trout reference samples, and only one haplotype was observed in all populations (C-48%). Sequence divergence among hatchery haplotypes ranged from 0.40%-3.0%. None of the haplotypes observed in McConaughy, Fish Lake, Eagle Lake, Arlee, Erwin or Shasta reference hatchery strains were observed in any of the redband trout populations from the Salmon and Snake rivers. Haplotype A, common throughout the Salmon and Snake river populations, was observed in the Hayspur Hatchery strain, although at low frequency (4%).

Table 3. Population, haplotype, sample size, and alphabetic designation of band patterns for each of the four restriction enzymes.

			Polymorphisms			
Population	**Haplotype**	**N**	**Hae-III**	**Hha-I**	**Hinf-I**	**Mse-I**
John Day Cr.	A	27	C	A	A	A
	B	2	C	A	A	C
Elk Cr.	A	26	C	A	A	A
	B	3	C	A	A	C
Hat Cr.	A	13	C	A	A	A
	B	16	C	A	A	C
Rattlesnake Cr.	A	13	C	A	A	A
	B	17	C	A	A	C
Wolf Cr.	A	29	C	A	A	A
Upper Rice Cr.	A	30	C	A	A	A
Divide Cr.	A	30	C	A	A	A
Falls Cr.	A	14	C	A	A	A
Upper Jersey Cr.	A	22	C	A	A	A
W.F. Crooked Cr.	A	12	C	A	A	A
	B	1	C	A	A	C
Reference Populations						
Rapid River	A	17	C	A	A	A
	B	1	C	A	A	C
	D	3	C	D	A	A
Hayspur	C	9	A	A	A	A
	E	13	C	C	C	B
	F	1	C	A	A	B
	A	1	C	A	A	A
	G	2	C	C	A	B
McConaughy	C	24	A	A	A	A
Fish Lake	C	12	A	A	A	A
	F	7	C	A	A	B
	E	4	C	C	C	B
	G	1	C	A	D	A
Eagle Lake	C	3	A	A	A	A
	H	21	B	B	B	B
Arlee	C	2	A	A	A	A
	E	16	C	C	C	B
	I	1	C	C	A	A
	J	1	C	A	C	A
Erwin	C	4	A	A	A	A
	E	16	C	C	C	B
Shasta	C	23	A	A	A	A
	H	1	B	B	B	B

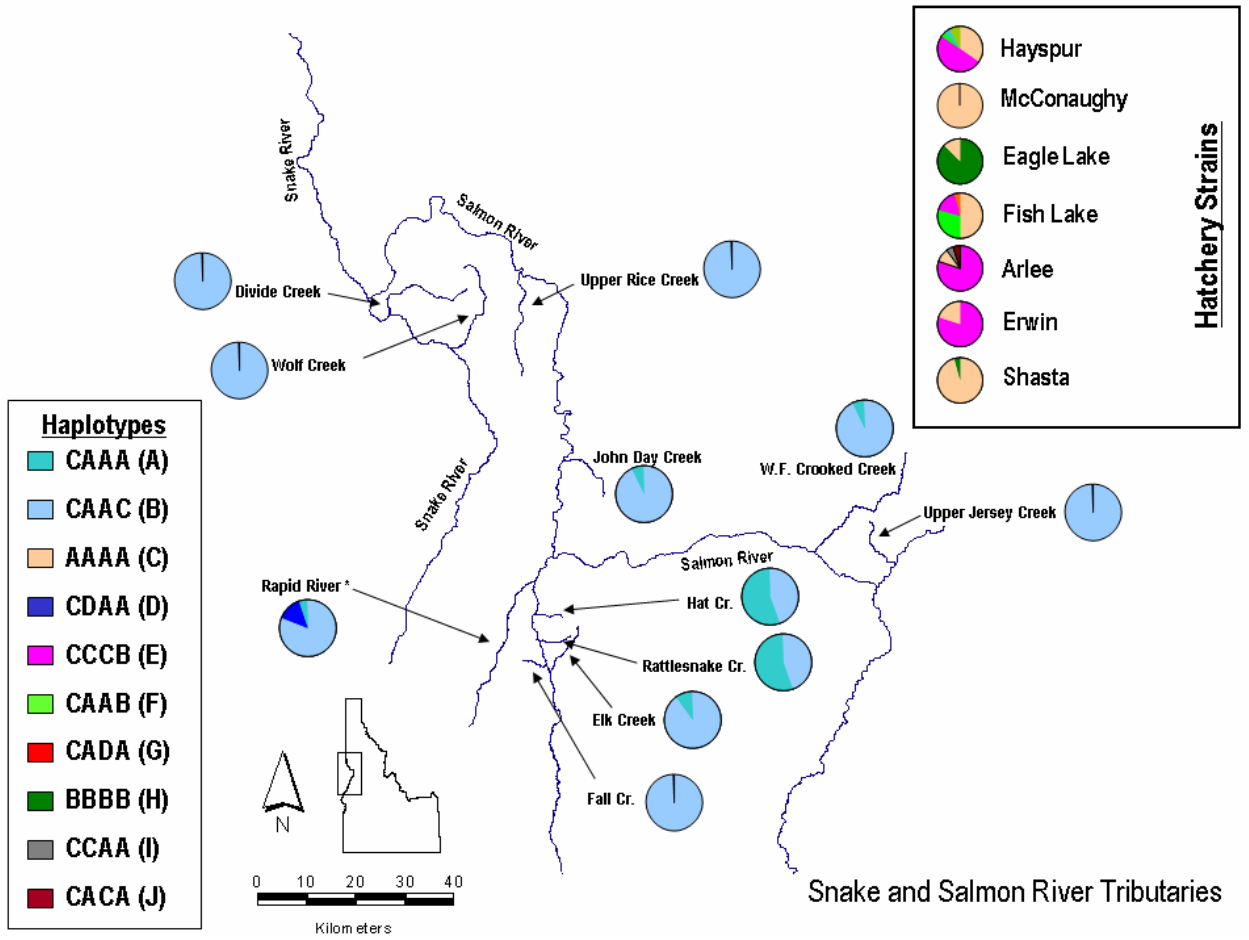

Figure 6. Distribution of mtDNA haplotypes in tributaries to the Snake and Salmon rivers, Idaho and reference hatchery rainbow trout strains.

Table 4. Percent sequence divergence matrix for observed haplotypes generated from REAP (McElroy et al. 1991).

Haplotypes	A	B	C	D	E	F	G	H	I
B	0.40								
C	0.41	0.82							
D	0.40	0.79	0.82						
E	1.61	2.00	2.07	2.00					
F	0.41	0.82	0.85	0.82	1.22				
G	0.82	1.22	1.26	1.22	0.79	0.41			
H	2.63	3.05	2.21	3.05	2.54	2.21	2.63		
I	0.40	0.79	0.82	0.79	1.18	0.82	0.40	3.05	
J	0.79	1.18	1.22	1.18	0.77	1.22	1.61	2.54	1.18

To depict relationships among the observed haplotypes, a dendogram (Figure 7) was constructed using the genetic distance estimates (Table 4) as input into the Kitsch program in PHYLIP (version 3.53). As expected, haplotypes A, B, and D (observed in samples from the Snake and Salmon river drainages) clustered together. Haplotype C, the most common haplotype observed in the hatchery reference populations, also clustered with haplotypes A, B, and D.

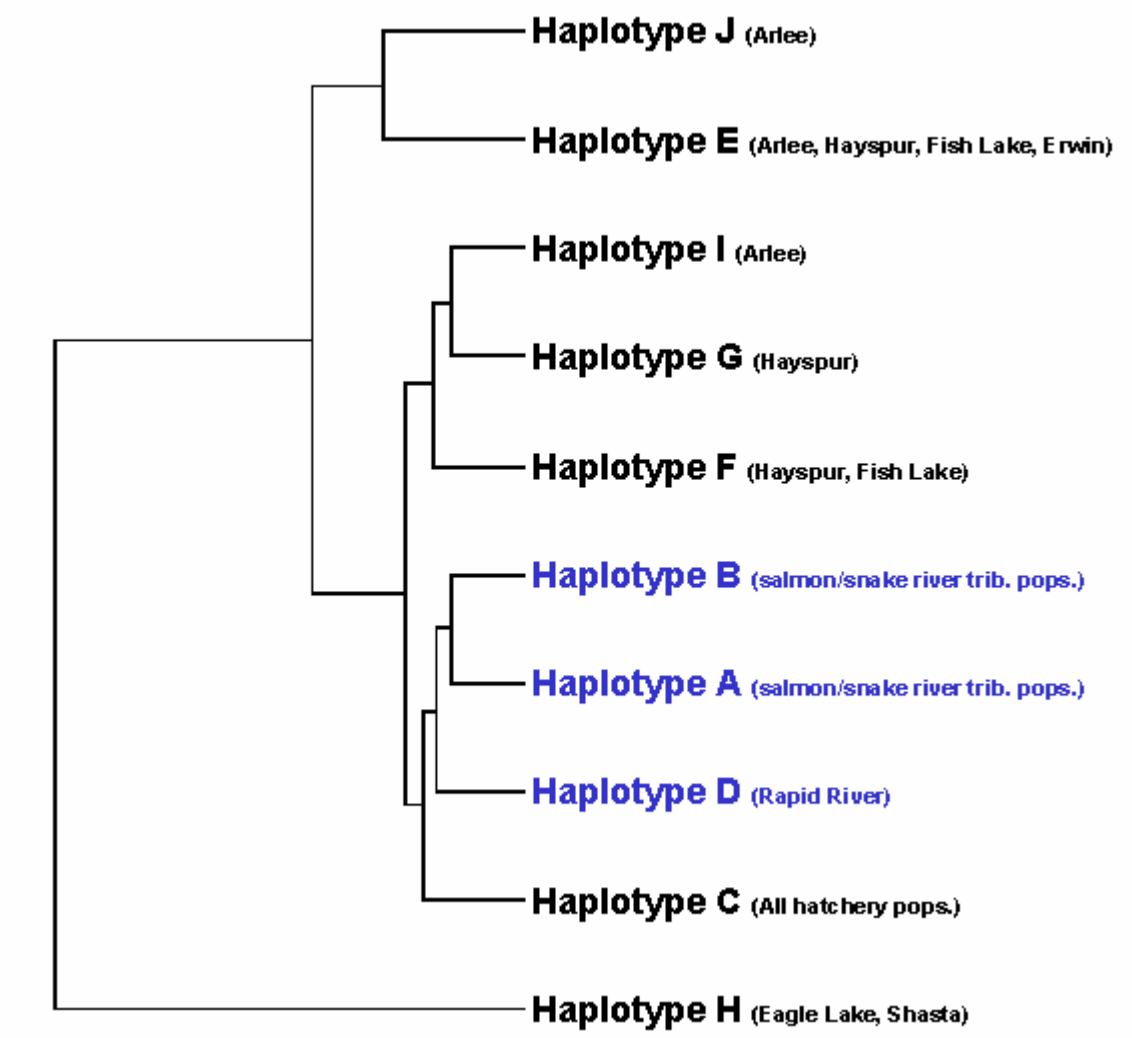

Figure 7. Unrooted Least Squares dendogram of observed mitochondrial haplotypes. Populations in which they were observed are in parentheses. Haplotypes B, A, and D were observed in redband samples from the Snake and Salmon river drainages.

DISCUSSION

The identification and conservation of pure, native redband trout populations are goals of both the Bureau of Land Management and the Idaho Department of Fish and Game. Both

agencies consider redband trout a “sensitive species” and have prioritized resources for their study and protection. Populations of redband trout found upstream of natural barriers are of particular conservation interest; they are protected from upstream invasion of exotic introduced trout, and they may make up an important component of the overall genetic diversity of *O. mykiss* in Idaho.

This current study of 10 populations of *O. mykiss* isolated upstream of migration barriers in the Snake and Salmon river drainages provides evidence that these populations are pure native interior redband trout. No mtDNA haplotypes observed in six reference hatchery rainbow trout populations/strains were observed in any of these populations. The predominant haplotypes observed in the Hayspur Hatchery population were also not observed in any of these allopatric populations. The haplotype observed in highest frequencies within these allopatric populations was observed in the Hayspur Hatchery population in low frequency (4%), likely because the Hayspur strain was partially founded from Idaho interior redband populations along with nonnative hatchery rainbow trout.

The question concerning whether these isolated populations should be placed in their own ESUs or DPSs independent from resident and anadromous *O. mykiss* populations below these barriers (Kostow 2003) is outside the scope of this limited study. This study does indicate that these populations, as well as one steelhead population from Rapid River, share mtDNA haplotypes. Previous research (Williams et al. 1996) has suggested that interior redband trout (east of the Cascade crest) are of monophyletic origin (they arose from one common ancestor). This limited mtDNA screen supports the idea that these isolated populations do not constitute unique lineages of their own, completely independent from other resident and anadromous *O. mykiss* populations within the Snake and Salmon river drainages. This is not to suggest, however, that these populations together represent one genetically homogeneous group. Depending on the length of time that these populations have been isolated from one another and from downstream populations, a microsatellite DNA screen could show substantial genetic divergence between populations (the result of a combination of forces including founding effects, genetic drift, and differential selective pressures).

The fact that these *O. mykiss* populations are naturally isolated by full passage barriers and that they appear to be pure redband trout (unaltered by any of the hatchery rainbow trout stocking that has taken place elsewhere in these drainages) suggests that they should be prioritized for conservation and likely managed independently from *O. mykiss* populations downstream of these barriers. Future research should include additional comparisons between populations upstream and downstream of barriers and should include a screen with microsatellite nuclear DNA markers to provide additional genetic information concerning the potential uniqueness and importance of these isolated *O. mykiss* populations.

JOB PERFORMANCE REPORT
SUBPROJECT #2: TESTING PHENOTYPE-BASED IDENTIFICATIONS OF WESTSLOPE CUTTHROAT TROUT, RAINBOW TROUT, AND HYBRIDS IN THE COEUR D'ALENE RIVER BASIN, IDAHO

State of: Idaho Grant No.: F-73-R-25, Fishery Research

Project No.: 2 Title: Native Species Investigations

Subproject #2: Testing phenotype-based identifications of westslope cutthroat trout, rainbow trout, and hybrids in the Coeur d'Alene River basin, Idaho

Contract Period: July 1, 2003 to June 30, 2004

ABSTRACT

Hybridization issues concerning westslope cutthroat trout and rainbow trout remained primary concerns for managers during the past year, and the Idaho Department of Fish and Game has continued to develop management strategies for reducing or eliminating hybrids and nonnative populations of rainbow trout. Many of these strategies rely on the ability of biologists and managers to use phenotype-based characters to distinguish accurately cutthroat trout from hybrids and rainbow trout. In this study, 68 *Oncorhynchus sp*. were randomly collected from four sites within the Coeur d'Alene River basin, Idaho. Every fish collected was photographed, recorded as cutthroat trout, rainbow trout, or hybrid, and sampled for genetic analysis (nonlethal fin clip). A diagnostic mitochondrial DNA marker and six diagnostic nuclear DNA markers were used to assign individual fish a genetic identification and test phenotypic calls. Results from this initial study suggest that some caution should be used when applying current phenotype-based procedures to distinguish westslope cutthroat trout, rainbow trout, and hybrids within the Coeur d'Alene River basin, Idaho. We failed to detect rainbow trout alleles within two fish phenotypically identified as "hybrid," and one sample phenotypically identified as a westslope cutthroat trout was genetically detected as a $>F_1$ hybrid. This demonstrates that current phenotype-based procedures will be unable to remove all hybrids from streams within the Coeur d'Alene River basin, Idaho. However, the results of this study indicate that current phenotype-based procedures could be used to reduce greatly the threat of hybridization and introgression within these streams without harming westslope cutthroat trout populations. All samples phenotypically identified as rainbow trout were genetically identified as rainbow trout or $>F_1$ hybrids. By removing all samples phenotypically identified as rainbow trout, researchers would have eliminated all of the rainbow trout in the sample and 60% of the genetically identified hybrids.

Authors:

Matthew Campbell, Fisheries Research Biologist

Christine Cegelski, Fisheries Genetics Research Assistant

INTRODUCTION

Introductions of rainbow trout *Oncorhynchus mykiss* for fisheries management purposes have occurred throughout the range of westslope cutthroat trout *O. clarkii lewisi* for more than 100 years. It has been well documented that these introductions have in some instances led to hybridization and introgression, a potentially serious, ongoing genetic hazard throughout much of the species present range (Weigel et al. 2002; Sage et al. 1992; Leary et al. 1984).

Current management strategies for reducing or eliminating hybrids and self-reproducing populations of introduced nonnative rainbow trout include changes to fishing regulations to allow for the harvest of hybrids and rainbow trout and the use of weirs on spawning tributaries to remove migrating hybrids and rainbow trout from spawning populations (Host 2003). Both of these strategies rely on the ability of biologists and fisherman to distinguish accurately cutthroat trout from hybrids and rainbow trout. Previous research on westslope cutthroat trout populations within the North Fork Clearwater basin indicated that a model using phenotypic characters could be used to identify accurately westslope cutthroat trout from hybrids and estimate the genetic status of populations within that basin (Weigel et al. 2002). Weigel et al. (2002) cautioned, however, that natural variation in phenotypic characters makes their use complicated and recommended genetic verification when applying phenotype-based procedures outside previously studied drainages.

The objective of this initial pilot study was to test the ability of field biologists to identify accurately westslope cutthroat trout, rainbow trout, and hybrids within the Coeur d'Alene River basin, Idaho using current phenotype-based procedures. The Coeur d'Alene River basin, Idaho was stocked from the late 1960s through the early 1990s with rainbow trout (http://www2.state.id.us/fishgame/fish/fishstocking/index.htm). Genetic studies performed in 1989, 1994, 1997, and 1998 documented rainbow trout hybridization and introgression throughout the Coeur d'Alene River basin, although the data suggested that in many of the sites examined, the level of hybridization was low and pure westslope cutthroat trout still existed (Coeur d'Alene Subbasin Summary 2001; http://www.cbfwa.org/files/province/mtncol/subsum/031601coeurdalene.pdf).

To test phenotype-based identifications, *Oncorhynchus sp*. were randomly collected from four sites within the Coeur d'Alene River basin, Idaho (Figure 8). Every fish collected was photographed, recorded as cutthroat trout, rainbow trout, or hybrid, and sampled for genetic analysis (nonlethal fin clip). A diagnostic mitochondrial DNA (mtDNA) marker and six nuclear DNA (nDNA) markers were used to assign individual fish a genetic identification.

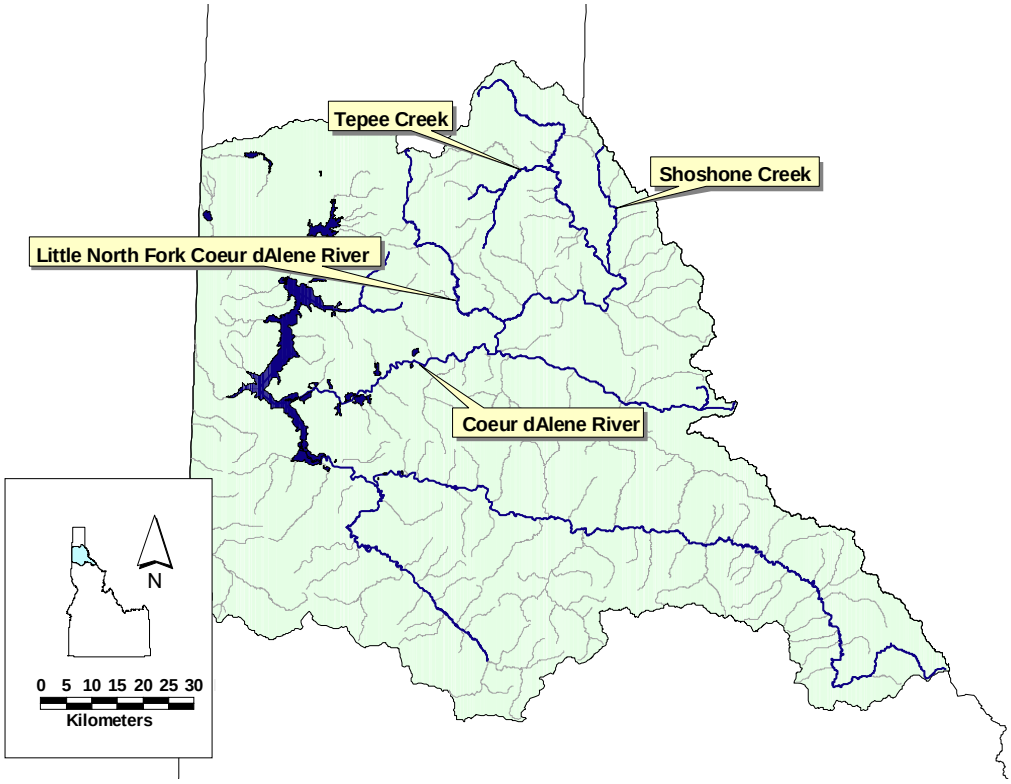

Figure 8. Map of four sample sites in Coeur d'Alene River basin, Idaho. Sample sizes are as follows: Shoshone Creek (N = 30), Tepee Creek (N = 15), Little NF Coeur d'Alene River (N = 18), and mainstem Coeur d'Alene River (N = 5).

OBJECTIVE

1. Test the ability of field biologists to identify accurately westslope cutthroat trout, rainbow trout, and hybrids within the Coeur d'Alene River basin, Idaho using current phenotype-based procedures.

METHODS

Genetic Analysis

Total genomic DNA was extracted from a 1 mm piece of fin clip following methods described by Paragamian et al. (1999) and adapted from protocols by Sambrook et al. (1989) and Hillis et al. (1996). Extracted DNA was resuspended in 100 µl TE. Restriction Fragment Length Polymorphism (RFLP) analyses were conducted using one mitochondrial DNA marker digested with *Hinf I* (Cytochrome b; Mays 2002) and one nuclear DNA marker digested with *Dde I* (Rag 3'; Campbell et al. 2002). Five simple sequence repeat (SSR) nDNA markers,

Occ35, Occ36, Occ38, Occ42, and OM55, diagnostic between rainbow trout and westslope cutthroat trout, were also amplified for each sample (Ostberg and Rodriguez 2002).

Digests were electrophoresed on 3% agarose gels and visualized as band patterns when fluoresced under UV-light (Figures 9 and 10). For the markers used in this study, “A” refers to a banding pattern unique to rainbow trout, whereas “B” refers to a banding pattern unique to westslope cutthroat trout. For the nDNA markers, the genotype “AA” refers to an individual that is homozygous for rainbow trout alleles, “BB” refers to an individual that is homozygous for westslope cutthroat trout alleles, and “AB” refers to an individual that is heterozygous with both a rainbow trout and westslope cutthroat trout allele. The letter designations for each of the seven markers used were later combined to infer if a sample was putatively pure or hybridized.

Hardy-Weinberg equilibrium (HWE) proportions were tested at each marker/restriction enzyme pair using the software program GENEPOP (Raymond & Rousset 1995) to assess if more than one population was sampled (e.g., sampled separate populations of westslope cutthroat trout and rainbow trout).

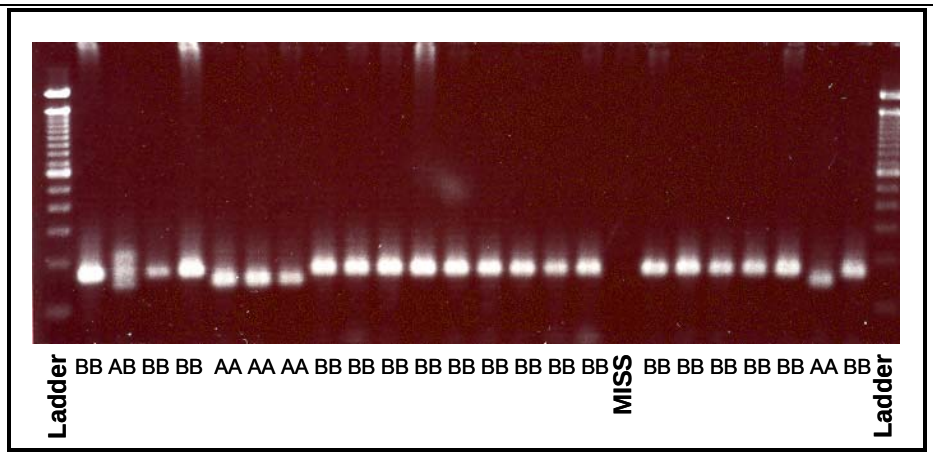

Figure 9. Photograph of 3% Synergel™ showing samples from Shoshone Creek, Coeur d’Alene River basin, Idaho. Locus shown is Occ38. “AA” refers to individuals that are homozygous for rainbow trout alleles, “BB” refers to individuals that are homozygous for cutthroat trout alleles, and “AB” refers to individuals that are heterozygous for both a rainbow trout allele and cutthroat trout allele.

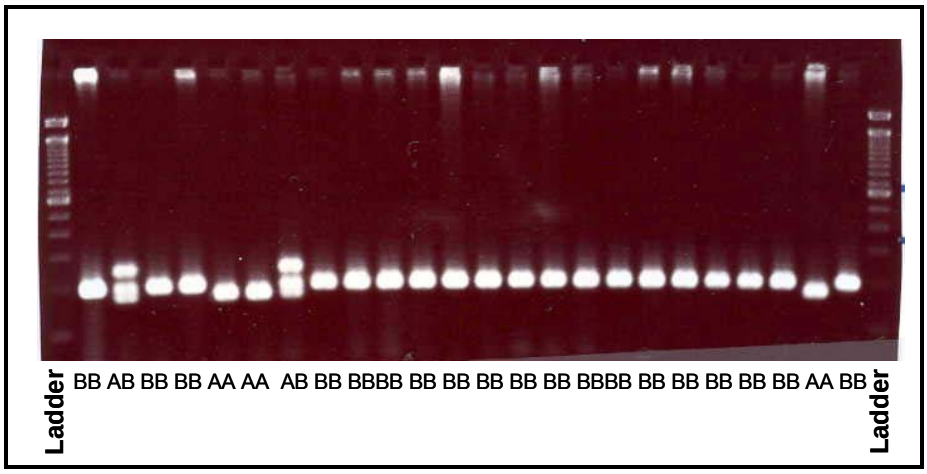

Figure 10. Photograph of 3% Synergel™ showing samples from Shoshone Creek, Coeur d'Alene River basin, Idaho. Locus shown is Occ42. "AA" refers to individuals that are homozygous for rainbow trout alleles, "BB" refers to individuals that are homozygous for cutthroat trout alleles, and "AB" refers to individuals that are heterozygous for both a rainbow trout allele and cutthroat trout allele.

RESULTS

In total, 68 samples were extracted for genetic analyses. Sixty-seven samples yielded sufficient DNA for PCR and RFLP analyses. Sample "CDA004" amplified for less than four markers and will need to be re-extracted in order to generate a complete genotype.

Of the 67 samples with complete genotypes (four or more markers), five samples with genotypes indicative of $>F_1$ hybrids ("SC0006," "SC0016," "SC0020," "SC0030," and "SC0038") were identified, and five samples with genotypes indicative of rainbow trout ("SC0009," "SC0011," "LNF0022," "CDA0002," and "CDA0003") were identified (Appendix 1). The remaining 57 samples had genotypes indicative of westslope cutthroat trout (Appendix 1). Of the samples that were identified as $>F_1$ hybrids, all of which came from Shoshone Creek, three had mitochondrial DNA of rainbow trout, and two had mitochondrial DNA of westslope cutthroat trout. All samples phenotypically identified as rainbow trout were genetically identified as either rainbow trout or $>F_1$ hybrids. Two fish, phenotypically identified as hybrids, had genotypes indicative of cutthroat, and one fish phenotypically identified as westslope cutthroat trout was genetically identified as a $>F_1$ hybrid.

A test for HWE was performed using the software program GENEPOP (Raymond and Rousset 1995). All six loci were significantly out of HWE ($p < .0001$) when tested on all samples. This suggests that we are not sampling one randomly mating population (a hybrid swarm), but rather that we are sampling westslope cutthroat trout, rainbow trout, and hybrids between the two. From these data it would appear that, despite a long history of rainbow trout stocking, there are likely some reproductive isolating mechanisms helping to limit hybridization and introgression between these two taxa (either pre- or post-isolating mechanisms) in some streams throughout the Coeur d'Alene River basin, Idaho.

DISCUSSION

Results from this initial pilot study suggest that some caution should be used when applying current phenotype-based procedures to distinguish westslope cutthroat trout, rainbow trout, and hybrids within the Coeur d'Alene River basin, Idaho. We failed to detect rainbow trout alleles within two fish phenotypically identified as "hybrid," and these fish were instead assigned the same genetic status as fish phenotypically identified as pure westslope cutthroat trout. A potential problem of misidentifying pure westslope cutthroat trout for hybrids is that we may unintentionally reduce natural phenotypic variability (and potentially genetic variability) present within these populations if we were to remove these individuals.

Another misidentification involved a sample that was phenotypically identified as westslope cutthroat trout while genetically detected as a $>F_1$ hybrid. This result is not particularly surprising, since previous research has demonstrated that westslope cutthroat trout with low levels of rainbow trout alleles may be phenotypically indistinguishable from pure westslope cutthroat trout (Leary et al. 1984). This does demonstrate, however, that current phenotype-based procedures will be unable to remove all hybrids from streams within the Coeur d'Alene River basin, Idaho.

On a positive note, it does appear as if current phenotype-based procedures could be used to reduce greatly the threat of hybridization and introgression within these streams, without harming westslope cutthroat trout populations. All samples phenotypically identified as rainbow trout were genetically identified as rainbow trout or $>F_1$ hybrids. By removing all samples phenotypically identified as rainbow trout, researchers would have eliminated all of the rainbow trout in the sample and 60% of the genetically identified hybrids.

Future research on westslope cutthroat trout populations in the Coeur d'Alene River basin, Idaho should assess whether phenotype-based identifications can be improved and determine what phenotypic characters are most useful in making accurate identifications.

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APPENDICES

Appendix A. Raw Data

			Cyt B					Rag 3'		
		PHENOID	Hae-III	Occ38	Occ42	OM 55	Occ35	Dde-I	Occ36	GENEID
Joe-03-1	SC0019	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-2	SC0016	RBT	A	AB	AB	BB	AA	AB	AA	>F1 HYB
Joe-03-3	SC0015	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-4	SC0013	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-5	SC0011	RBT	A	AA	AA	AA	AA	AA	AA	RBT
Joe-03-6	SC0009	RBT	A	AA	AA	AA	AA	AA	AA	RBT
Joe-03-7	SC0006	RBT	A	AA	AB	AB	AB	AB	AB	>F1 HYB
Joe-03-8	SC0005	CUT	B	BB	BB	BB	MISS	BB	BB	CUT
Joe-03-9	SC0003	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-10	SC0028	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-11	SC0027	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-12	SC0026	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-13	SC0025	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-14	SC0024	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-15	SC0023	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-16	SC0022	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-17	SC0021	CUT	C	MISS	BB	BB	BB	BB	BB	CUT
Joe-03-18	SC0020	CUT	A	BB	BB	BB	BB	BB	AB	>F1 HYB
Joe-03-19	SC0043	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-20	SC0042	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-21	SC0040	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-22	SC0039	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-23	SC0038	RBT	C	AA	AA	AA	AB	AA	AB	>F1 HYB
Joe-03-24	SC0036	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-25	SC0031	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-26	SC0030	CUT	C	AB	AB	BB	BB	BB	BB	>F1 HYB
Joe-03-27	SC0029	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-28	TP0007	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-29	TP0006	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-30	TP0004	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-31	TP0003	CUT	C	BB	MISS	BB	BB	BB	BB	CUT
Joe-03-32	TP0002	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-33	TP0001	HYB	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-34	SC0046	CUT	MISS	BB	BB	BB	BB	MISS	BB	CUT
Joe-03-35	SC0045	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-36	SC0044	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-37	TP0008	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-38	TP0009	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-39	TP0010	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-40	TP0011	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-41	TP0012	CUT	MISS	BB	MISS	BB	BB	BB	BB	CUT
Joe-03-42	TP0013	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-43	TP0014	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-44	TP0015	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-45	TP0018	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-46	LNF0022	RBT	A	AA	AA	AA	AA	AA	AA	RBT
Joe-03-47	LNF0025	HYB	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-49	LNF0002	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-50	LNF0003	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-51	LNF0004	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-52	LNF0006	CUT	C	BB	BB	BB	BB	BB	BB	CUT

Appendix A. Continued.

			Cyt B					Rag 3'		
		PHENOID	**Hae-III**	**Occ38**	**Occ42**	**OM 55**	**Occ35**	**Dde-I**	**Occ36**	**GENEID**
Joe-03-53	LNF0007	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-54	LNF0009	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-55	LNF0010	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-56	LNF0011	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-57	LNF0012	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-58	LNF0014	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-59	LNF0020	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-60	LNF0017	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-61	LNF0020	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-62	LNF0021	CUT	B	BB	BB	BB	BB	BB	BB	CUT
Joe-03-63	LNF0023	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-64	LNF0024	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-65	CDA001	CUT	C	BB	BB	BB	BB	BB	BB	CUT
Joe-03-66	CDA002	RBT	A	AA	AA	AA	AA	AA	AA	RBT
Joe-03-67	CDA003	RBT	A	AA	AA	AA	AA	AA	AA	RBT
Joe-03-68	CDA004	CUT	B	MISS	MISS	MISS	MISS	MISS	MISS	MISS
Joe-03-69	CDA005	CUT	C	BB	BB	BB	BB	BB	BB	CUT

*Joe-03-48 was a brook trout

Prepared by:

Matthew Campbell
Sr. Fisheries Research Biologist

Christine Cegelski
Fisheries Genetics Research Assistant

Approved by:

IDAHO DEPARTMENT OF FISH AND GAME

Virgil K. Moore, Chief
Bureau of Fisheries

Daniel J. Schill
Fisheries Research Manager